Antimicrobial resistance of bacteria isolated from patients with bloodstream infections at a tertiary care hospital in the Democratic Republic of the Congo

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Background. Bloodstream infection (BSI) is a life-threatening condition that requires rapid antimicrobial treatment.
Methods. We determined the prevalence of bacterial isolates associated with BSI at Bukavu General Hospital (BGH), South Kivu Province, Democratic Republic of the Congo, and their patterns of susceptibility to antimicrobial drugs, from February 2013 to January 2014.
Results. We cultured 112 clinically relevant isolates from 320 blood cultures. Of these isolates, 104 (92.9%) were Gram-negative bacteria (GNB), with 103 bacilli (92.0%) and one coccus (0.9%). Among GNB, *Escherichia coli* (51.9%), *Klebsiella* spp. (20.2%), *Enterobacter* spp. (6.7%), *Shigella* spp. (5.8%) and *Salmonella* spp. (4.8%) were the most frequent agents causing BSIs. Other GNB isolates included *Proteus* spp., *Citrobacter* spp. and *Pseudomonas aeruginosa* (both 2.9%), and *Acinetobacter* spp. and *Neisseria* spp. (both 0.9%). High rates of resistance to co-trimoxazole (100%), erythromycin (100%) and ampicillin (66.7 - 100%) and moderate to high resistance to ciprofloxacin, ceftazidime, ceftriaxone, cefuroxime and cefepime were observed among GNB. Furthermore, there were high rates of multidrug resistance and of extended-spectrum β-lactamase (ESBL) production phenotype among Enterobacteriaceae. Gram-positive bacteria included three *Staphylococcus aureus* isolates (2.7%), four oxacillin-resistant coagulase-negative staphylococci (CoNS) isolates (3.6%) and one *Streptococcus pneumoniae* (0.9%). No oxacillin-resistant *S. aureus* was isolated. Among clinically relevant staphylococci, susceptibility to co-trimoxazole and ampicillin was low (0 - 25%). In addition, 58 contaminant CoNS were isolated from blood cultures, and the calculated ratio of contaminants to pathogens in blood cultures was 1:2.
Conclusions. Multidrug-resistant and ESBL-producing GNB are the leading cause of BSI at BGH.

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Bloodstream infection (BSI) refers to the presence of microbial pathogens in the blood. These are present as a result of infection, not specimen contamination.[1-3] BSI is a major cause of community and healthcare-associated infections and is associated with high mortality.[3-7]

Infectious diseases are the leading cause of death in the Democratic Republic of the Congo (DRC).[8] In fact, the DRC has a crude mortality rate well above the average for sub-Saharan countries[9] and the highest under-5 mortality rate in Africa,[10] with malaria, pneumonia and diarrhoea the leading causes of death.[11,12] In South Kivu Province, most healthcare facilities lack the capacity to identify causative agents of infectious diseases reliably, including invasive bacterial infections such as BSIs. BSI and malaria are practically indistinguishable on clinical examination,[13] and available World Health Organization (WHO) guidelines for managing childhood illnesses fail to identify up to half of the cases of BSI.[14] In view of the fact that fever – a symptom common to malaria and BSI – is the most frequent reason for presentation to hospital in most developing countries,[5,15] the development of laboratory capacity for identification of pathogens is critical in order to improve the outcome of febrile diseases and enable rapid differential diagnosis between malaria and BSI.[16,17] We report the results of our first year's experience using blood cultures in the laboratory of Bukavu General Hospital (BGH), South Kivu Province, in order to identify pathogens involved in BSIs. This report follows on from a previous study that documented a high rate of antimicrobial drug-resistant isolates in patients with urinary tract infections at the same healthcare facility.[18]

Methods

Study design

This cross-sectional study was conducted in inpatients in various wards of BGH who were suspected of having BSI. This hospital has 385 beds, handles 6 400 admissions and 4 900 outpatients per year, and is one of the main healthcare facilities in Bukavu, a city of more than 500 000 inhabitants in South Kivu Province in eastern DRC.

Laboratory methods

BAC/ALERT FA FAN Aerobic and BAC/ALERT FA FAN Anaerobic blood culture bottles and BAC/ALERT PF Pediatric FAN (Biomérieux, Belgium) were dispatched to various wards of the hospital. The decision to perform blood culture was based on the following criteria: fever (temperature >38.0°C), a negative thick blood smear for detection of *Plasmodium* spp., and a white blood cell count of >10 × 10^9/L with >70% neutrophils. Nurses were instructed to sample blood during a febrile episode. For sampling, the patient's skin was cleaned with 70% ethanol and allowed to dry prior to venous puncture. Blood was collected using strict aseptic technique, and approximately 10 mL

for adult patients was immediately inoculated into the BAC/ALERT FA FAN Aerobic and BAC/ALERT FA FAN Anaerobic blood culture bottles. In the case of children, 2 - 5 mL of blood was inoculated in a BAC/ALERT PF Pediatric FAN. The inoculated vials were incubated straight away at 37°C for up to 7 days. The vials were checked visually every day to detect any colour change at the bottom of the bottle, from a blue-green to a yellow colour. For vials displaying a positive signal, cultures were Gram-stained and subcultures performed on 5% sheep blood agar plates for staphylococci. For *Streptococcus*, a disc of optochin was added to the streaked blood agar plate and incubation was carried out at 37°C in a 5% CO_2 atmosphere. Subculture of Gram-negative bacilli was performed on MacConkey agar, whereas Gram-negative cocci were subcultured on chocolate agar. Standard biochemical methods were subsequently used to identify bacteria at the species level.[19]

Antimicrobial susceptibility tests were performed using the disc diffusion method on Mueller-Hinton agar II (Bio-Rad, Nazareth Eke, Belgium), and the results were interpreted according to the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2015).[20] Antibiotic discs were purchased from Bio-Rad (Nazareth Eke). For isolates of staphylococci, testing of antimicrobial drug susceptibility to ampicillin, amikacin, clindamycin, erythromycin, gentamicin, oxacillin and vancomycin was performed. In order to assess the antimicrobial drug susceptibility pattern of Gram-negative isolates, ampicillin, gentamicin, amoxicillin-clavulanic acid, ceftriaxone, cefuroxime, ceftazidime, cefepime, imipenem, amikacin, ciprofloxacin, erythromycin and clindamycin were tested. Isolates showing resistance to at least one cephalosporin were tested for extended-spectrum β-lactamase (ESBL) production by the double-disc synergy test on Mueller-Hinton agar using ceftazidime and ceftriaxone placed at a distance of 20 mm from a disc containing amoxicillin plus clavulanic acid. A clear-cut enhancement of the inhibition in front of either ceftazidime and ceftriaxone discs towards the clavulanic acid-containing disc (also called 'champagne cork' or 'keyhole') was interpreted as positive for ESBL production.[21] E-test strips (BioMérieux, France) were used for confirmation of ESBL production. Minimum inhibitory concentrations of cefotaxime and ceftazidime, with and without clavulanic acid, were determined after 16 - 18 hours' incubation on Mueller-Hinton plates inoculated with suspension of isolates at a fixed density (0.5 - 0.6 McFarland standard). *Escherichia coli* ATCC 35218 and *Klebsiella pneumoniae* ATCC 700603 strains were used as ESBL-negative and positive controls, respectively. Multidrug resistance (MDR) was defined as non-susceptibility to at least one agent in three or more antimicrobial categories.[16] All MDR isolates were cryopreserved at –80°C for further studies, with the exception of methicillin-resistant coagulase-negative staphylococci (CoNS), all CoNS being considered contaminants.

Statistical analysis

Statistical analyses were performed using the SPSS statistical package release 12.0 for Windows (SPSS, USA). Comparisons of proportions of bacteria isolated and antimicrobial susceptibility results were analysed using the χ^2 test. The level of significance was $p<0.05$.

Ethics

Ethical approval was granted by the Ethical Committee of the Université catholique de Bukavu, DRC, and the DRC Ministry of Health. The study complies with WHO and international guidelines (European Society of Clinical Microbiology and Infectious Diseases Study Group for Antimicrobial Resistance Surveillance and Clinical Laboratory Standards Institute) on antibiotic surveillance, for which no recommendation for an informed consent has been issued. The diagnostic procedure (blood cultures) is part of the standard diagnostic work-up of patients suspected of having bacteraemia. Clinical information as presented, and information about use of antibiotics, was the standard information on the laboratory request form. Data were reviewed and analysed anonymously.

Results

A total of 320 blood samples from 320 different patients were cultured. The mean age of the patients was 21.6 years (range 0 - 75), and 162 were males and 158 females. Bacterial pathogens were detected in 170 cultures, 29.4% of which were from children (<17 years). The distribution and percentages of the various bacterial isolates are shown in Table 1.

Isolates of Gram-negative bacteria (GNB) (104, 61.2%) were significantly more prevalent than Gram-positive isolates (66, 38.8%) ($p<0.05$). Among the Gram-positive isolates, the predominant isolate was CoNS ($n=62$); of these, 58 were susceptible to oxacillin. Four CoNS isolates displayed low susceptibility to oxacillin and were accordingly considered methicillin-resistant CoNS. *Staphylococcus aureus* was represented by three isolates, none resistant to oxacillin. Only one *S. pneumoniae* was isolated, from a blood sample of a 3-year-old girl; this isolate was resistant to erythromycin and amikacin, while retaining susceptibility to gentamicin. The Gram-negative isolates were overwhelmingly represented by Enterobacteriaceae (100/104 isolates). Enterobacteriaceae included *E. coli* (48.6%), *Klebsiella* spp. (18.9%) and *Enterobacter* spp. (6.3%). Rare Enterobacteriaceae included *Shigella* spp., *Salmonella* spp, *Citrobacter* spp. and

Table 1. Distribution of isolates in blood cultures at BGH, February 2013 - January 2014

Bacterial isolate	Isolates, *n*	% of total isolates (*N*=170)	% of BSI pathogens (*N*=112)
Escherichia coli	54	31.8	48.2
Klebsiella spp.	21	12.4	18.3
Enterobacter spp.	7	4.1	6.3
Shigella spp.	6	3.5	5.4
Salmonella spp.	5	2.9	4.5
Citrobacter spp.	3	1.8	2.7
Pseudomonas aeruginosa	3	1.8	2.7
Proteus spp.	3	1.8	2.7
Acinetobacter spp.	1	0.6	0.9
Neisseria spp.	1	0.6	0.9
Staphylococcus aureus	3	1.8	2.7
Methicillin-resistant CoNS	4	2.4	3.6
Streptococcus pneumoniae	1	0.6	0.9
Methicillin-susceptible CoNS (contaminants)	58	34.1	-
Total	170	100	100

Table 2. Antimicrobial resistance (%) in 104 Gram-negative blood isolates collected at BGH, February 2013 - January 2014

Antimicrobial drug	*Escherichia coli* (*n*=54)	*Klebsiella* spp. (*n*= 21)	*Enterobacter* spp. (*n*=7)	*Citrobacter* spp. (*n*=3)	*Salmonella* spp. (*n*=5)	*Shigella* spp. (*n*=6)	*Proteus* spp. (*n*=3)	*Acinetobacter* spp. (*n*= 1)	*Pseudomonas aeruginosa* (*n*=3)	*Neisseria* spp. (*n*=1)
Amikacin	5.6	4.8	0	0	0	0	66.7	0	0	0
Ampicillin	98.1	95.2	100.0	100.0	100.0	83.3	66.7	100	100	NT
Amoxicillin/ clavulanate	7.4	19.0	14.2	33.3	0	50.0	0	100	100	NT
Cefepime	13.0	91	57.1	33.3	0	83.3	66.7	NT	NT	NT
Ceftazidine	46.3	52.4	14.2	66.7	20	83.3	66.7	100	100	NT
Ceftriaxone	24.1	19.0	28.5	33.3	80	83.3	0	100	NT	NT
Cefuroxine	81.5	85.7	85.6	66.7	80	100	100	100	66.7	NT
Ciprofloxacin	31.5	33.3	57.1	0	20	33.3	66.7	0	66.7	100
Erythromycin	100	100	100	100	100	100	100	100	100	NT
Gentamicin	7.4	28.6	14.2	0	100	100	66.7	0	0	NT
Imipenem	0	4.8	0	0	0	0	0	0	0	NT
Co-trimoxazole	100	100	100	100	100	100	100	100	100	100
MDR phenotype	20.4	47.6	57.1	66.7	0	66.7	0	100	100	0
ESBL-producing phenotype	16.7	47.6	28.5	33.3	0	0	0	NT	NT	NT

NT = not tested.

Proteus spp. The four non-Enterobacteriaceae Gram-negative isolates included three *Pseudomonas aeruginosa*, one *Acinetobacter* spp. and one *Neisseria* spp.

Tables 2 and 3 show the antimicrobial susceptibility patterns of Gram-negative and Gram-positive bacterial isolates. With the exception of the two *Proteus* spp., Gram-negative isolates were often susceptible to amikacin, gentamicin and ciprofloxacin. The MDR phenotype was present in 36 (34.6%) Gram-negative isolates, with >50% also displaying an ESBL-production phenotype. Indeed, 82% MDR *E. coli* isolates were also ESBL producers, whereas 100% of *Klebsiella* spp. isolates displayed the ESBL-production phenotype. Of note, all Gram-negative bacilli displayed low susceptibility to co-trimoxazole, ampicillin and erythromycin. Worryingly, two MDR and ESBL-producing *Klebsiella* spp. isolates also displayed resistance to imipenem, one of the few carbapenems available in the province.

Nitrofurantoin, an oral drug displaying satisfactory activity against uropathogens in South Kivu,[18] was not included in the antimicrobial panel because only parenteral antimicrobials were administered for the treatment of BSIs.

Table 3. Antimicrobial resistance (%) in 8 Gram-positive blood isolates collected at BGH, February 2013 to January 2014

Antimicrobial drug tested	*Staphylococcus aureus* (3 isolates)	CoNS (4 isolates)	*Streptococcus pneumoniae* (1 isolate)
Amikacin	0	0	100.0
Ampicillin	100	75.0	NT
Sulphamethoxazole + trimethoprim	100	100	NT
Clindamycin	0	0	NT
Gentamicin	0.00	50.0	0
Erythromycin	33.3	0	0
Oxacillin	0	10.0	NT
Vancomycin	0	0	NT

NT = not tested.

Discussion

BSI is associated with high mortality and high healthcare costs, especially when the bacteria have low susceptibility to antimicrobial drugs.[22] Accordingly, the implementation of laboratory capacity for specific diagnosis of causative bacterial agents and determination of their antimicrobial susceptibility profile is pivotal in curbing mortality related to BSI. Although blood cultures have been performed at our hospital for many years, they were characterised by a very low yield of positive isolates, with unreliable identification resulting in largely empirical treatment of presumptive BSI. Changes in the blood culture process in the past year have resulted in a spectacular rise in positive blood cultures in patients with suspected BSI. Despite these encouraging improvements, blood culture at our hospital is still plagued by a high rate of contaminant CoNS, when compared with benchmarks in the field.[23,24] Action must therefore be taken to improve skin decontamination prior to blood sampling; the low susceptibility of CoNS to oxacillin needs further confirmation, as it has been shown that disc testing is not an accurate method for the determination of methicillin susceptibility of CoNS.[25]

In our study, 32.5% of blood cultures yielded significant positive growth. This rate

is high when compared with rates in other African countries.[22,26-28] We found a high prevalence of MDR GNB as major causative agents of BSI.

Among GNB, Enterobacteriaceae, particularly *E. coli*, were the most frequently isolated pathogens. Our results diverge from those previously reported in this province more than a decade ago that documented *Salmonella* spp. as the leading cause of bacteraemia in a rural paediatric hospital.[5] Oddly, Gram-positive bacteria did not play an important role in BSI when compared with other studies in developing countries;[15,27,29,30] in fact, only three *S. aureus* and one *S. pneumoniae* were isolated. Given this steadily growing danger of MDR and ESBL-producing isolates in South Kivu, as underlined by our findings, a strict antibiotic policy should be implemented urgently in the province with an emphasis on local susceptibility findings. Another important issue in the province is the lack of regulation regarding prescription of antibiotics, which are widely used, even for minor illnesses such as rhinitis. Whereas no study has assessed this phenomenon, it is worth noting that a recent study documented a high level of irrational prescription of antibiotics by healthcare professionals in the Orientale Province of DRC,[31] and that this practice is often associated with a steady increase of antimicrobial drug resistance in low-income countries. The identification of two carbapenem-resistant *Klebsiella* spp. isolates in the province is consistent with a recent observation of a carbapenem-resistant *Enterobacter* spp. in a urinary tract infection in the same province.[18] The emergence and possible spread of carbapenem-resistant Enterobacteriaceae isolates in the province would represent an additional step in the wrong direction that might be enhanced by misuse of carpabenems, as observed a few years ago in some Asian countries.[32]

Study limitations

Our study has several limitations. We analysed only 320 blood cultures, a low number that might result in selection bias. Rates obtained during this study should therefore be interpreted with caution. Whereas no patient reported having HIV infection, no objective data were available regarding HIV status of BSI patients.

Conclusion

Although performed on a limited set of blood cultures, our study underscores the high prevalence of MDR-resistant bacteria responsible for BSI at BGH. The high rate of MDR ESBL-producing GNB is consistent with a previous study performed on isolates from urinary tract infections at the same hospital. Accordingly, these findings should compel provincial healthcare stakeholders to take immediate action aimed at tackling this dangerous trend, before the situation slips beyond any possible repair. These actions should include the establishment of guidelines for prescription of antimicrobial drugs and the setting up of antimicrobial drug control in the province.

Author contributions. LMI, LK and RBC participated in the design of the study, LMI oversaw the data collection, LMI and LK were responsible for the laboratory assays, and FBK, MI and PNM contributed to implementation of blood culture assays. LMI wrote the first draft of the article, and all the authors participated in the manuscript revision. All the authors read and approved the final manuscript.

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